Species-Specific Induction of Angiotensinogen mRNA in Transgenic Mouse Liver during Acute Phase Reaction

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Acute phase reaction is a systemic response accompanying an inflammatory response caused by tissue injury, infection, or neoplasia [4]. During the acute phase response, expression of a heterogeneous group of proteins synthesized preferentially in the liver, collectively called acute phase proteins, is altered. Since the acute phase response is biologically significant and represents an excellent system for studying how gene expression is regulated in fully differentiated tissue in response to external stimuli, expression of the acute phase protein genes has been studied in several species by using whole animals, primary hepatocytes in culture, and a series of animal and human hepatoma cell lines.

Angiotensinogen is a protein precursor of angiotensin II, a biologically active octapeptide hormone that plays an important role in the regulation of blood pressure and electrolyte balance [13]. Although angiotensinogen is expressed in multiple tissues, the liver is a major source of plasma angiotensinogen. The circulating amounts of angiotensinogen is thought to be rate limiting in the formation of angiotensin II [13]. Because of the limited capacity of the hepatocyte to store presynthesized proteins and regulate their secretion, changes in the amount of circulating angiotensinogen in response to a number of external stimuli may be regulated by gene transcription. Indeed, level of angiotensinogen mRNA in rat liver changes in response to multiple hormones [1]. Furthermore, it has been reported that rat angiotensinogen mRNA in the liver increased by the administration of bacterial endotoxin, lipopolysaccharide (LPS) [9] and that level of its proteins ecretion from rat hepatoma cell line (Reuber H35) to the culture medium was augmented by interleukin 6 (IL-6), a physiological acute phase reactant [8].

It has been reported that expression pattern of the acute phase proteins varies from one species to another: Serum amyloid P, for example, shows the dramatic elevation during the acute phase in mouse [12] but not in rat or human [6]; α_2-macroglobulin is the acute phase protein with the most spectacular changes in rat but not in human [7], suggesting that synthesis of acute phase proteins are controlled by a species-specific fashion during the acute phase response. However, little is known about interspecies differences in the acute phase responsivity of the angiotensinogen gene expression. In the present report, we have therefore investigated changes in hepatic mRNA for the endogenous and introduced angiotensinogen genes during the acute phase response, using the transgenic mice (C57BL/6) with the 14-kb human angiotensinogen gene containing the 1.3-kb 5'-flanking promoter sequences with sufficient genetic information necessary for the dominant-hepatic expression [15]; the transgene consists of 5 exons interrupted by 4 introns [5].

In order to distinguish the mouse angiotensinogen mRNA sequence from the foreign one in the transgenic mice by Northern blot analysis, we have first prepared the differential DNA probes. The DIAGON computer program [14] was used to compare the cDNA sequences of the two species [3, 9] and to obtain a clear representation of the pattern of homology (Fig. 1). As shown by a long main diagonal lines of dots, quite striking homology commences at the start site of transcription and continue to nucleotide position at 1470; thereafter, a dramatic

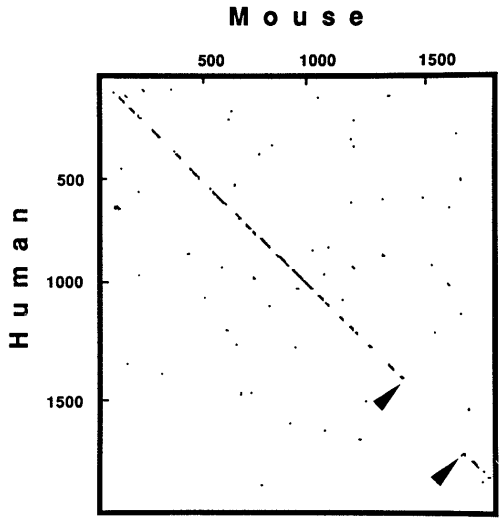

Fig. 1. Dot matrix comparison of the cDNA sequences of mouse (horizontal axis) and human (vertical axis) angiotensinogens. The numbering along the axes corresponds to the nucleotide position in the cDNAs. The mouse sequence was aligned for maximal match with the human sequence using the DIAGON program. The parameters used were a span length of 15 and a proportional matching score of 12. Diagonal lines indicate homologous regions. The DNA regions used for Northern blot analysis as the differential probes are shown by arrowheads.

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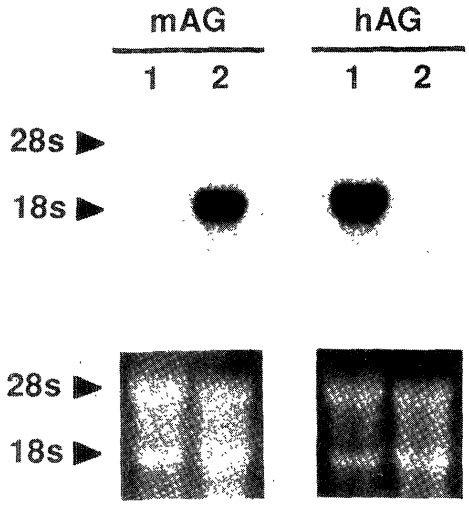

Fig. 2. Northern blot analysis of angiotensinogen mRNA. The upper panels show the blots of total RNAs (10 μg/lane) from human hepatoma cell line HepG2 (lane 1) and mouse liver (lane 2) hybridized to mouse (mAG) or human (hAG) angiotensinogen probes. The membrane was exposed for 24 hr. The lower panels show the ethidium bromide-stained ribosomal RNA (rRNA) bands prior to transfer to document RNA quantitation. The positions of 28S and 18S rRNA markers are indicated.

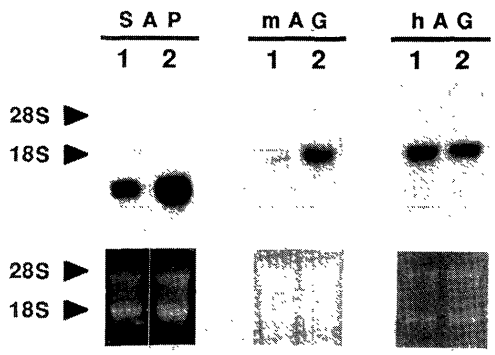

Fig. 3. Effect of LPS in combination with glucocorticoid on accumulation of angiotensinogen mRNA in the transgenic mouse liver. The upper panels show the blots of total RNAs (left panel, 10 μg/lane; middle, 10 μg/lane and right, 2 μg/lane) hybridized to mouse serum amyloid A (SAP), mAG, and hAG probes. The membrane was exposed for 6 hr. The ethidium bromide-stained rRNAs are shown in the lower panels to indicate relative amount of RNA (lane 1, untreated transgenic mouse liver and lane 2, stimulated transgenic mouse liver). The positions of 28S and 18S RNA markers are indicated.

reduction in sequence homology is observed, as indicated by arrowheads. We therefore chose and excised these DNA regions for hybridization probes from the mouse [16] and human [5] angiotensinogen genes; 381-bp *Aat*I-*Aat*II and 298-bp *Apa*I-*Eco*RI DNA fragments for mouse and human, respectively.

In order to confirm specificities of these probes, total RNA extracted from nontransgenic mouse (C57BL/6) liver and human hepatoma HepG2 cells that express a considerable quantity of angiotensinogen mRNA [5] was denatured, separated on 1.2% agarose gel, and transferred to GeneScreen Plus nylon membrane. Two sets of the membrane were then hybridized to each ^{32}P-labeled DNA probe as described previously [5]. As shown in Fig. 2, the mouse probe did hybridize to the mouse angiotensinogen mRNA but not to the human mRNA and the human probe specifically detected human angiotensinogen mRNA but not cross-hybridized to the mouse one. This indicates that these species-specific DNA probes are available for further investigation of the angiotensinogen gene expression in the transgenic mice by Northern blot analysis.

The acute phase response of the liver genes has been experimentally studied in whole animals, such as rat or mouse by injection with LPS. In order to examine whether levels of angiotensinogen mRNA is elevated during the acute phase response in the transgenic mice, acute inflammation was induced in groups of two transgenic mice (20–22 g body weight) by a single intraperitoneal injection of LPS (1 μg/g body weight). Glucocorticoid (Gc) (7 μg/g body weight) was used in combination with LPS for activating gene expression, since Gc was found to enhance the induction of acute phase protein gene [11]. After inflammatory stimuli for 6 hr, mice were killed by cervical dislocation, and liver was removed and frozen immediately in liquid nitrogen. Total RNA extracted from the liver of unstimulated and stimulated transgenic mice was subjected to Northern blot analysis with the species-specific angiotensinogen probes. Mouse serum amyloid P (SAP) cDNA was used as a control probe for the acute phase reaction, because level of SAP mRNA was shown to be induced by the administration of LPS [12].

Although Gc had no effect on the induction of SAP mRNA, LPS increased the mRNA level significantly (data not shown). On the other hand, mouse angiotensinogen and the transgene mRNAs were not induced by LPS or Gc alone (data not shown). As displayed in Fig. 3, however, the synergistic effect of LPS and Gc on increase in mouse angiotensinogen mRNA level was observed, and SAP mRNA was also induced by both agents to levels comparable to that in the liver treated with LPS alone. Unexpectedly, neither agents induced the transgene mRNA in the mouse liver synergistically. This was consistent with our initial observation that IL-6 or Gc alone, or both do not increase angiotensinogen mRNA level in HepG2 cells (data not shown). Similarly, the transcription rate of a reporter gene with the 1.3-kb transgene promoter containing a possible acute phase-responsive sequence [5] was not activated by IL-6 or Gc alone, or both in transfected HepG2 cells (unpublished

results). Therefore, these preliminary *in vitro* observations may support the current results that the foreign gene in the transgenic mouse liver is not induced under the present experimental conditions.

Although several investigations demonstrated that expression of the endogenous and introduced acute phase protein genes including α_1-acid glycoprotein [2] are simultaneously induced by an inflammatory reaction in the transgenic mouse liver, the present study provided evidence that the endogenous and foreign angiotensinogen genes are differentially regulated during the acute phase response in the transgenic mice. Because of these characteristic patterns of expression, the angiotensinogen gene may become an interesting model for investigation of regulatory mechanisms that determine species specificity.

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